

What is claimed is:

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1. A method for quantitatively assaying one or more target molecules in a first sample, comprising:

5 (a) adding to the first sample, a preparation of a nucleic acid aptamer specific for each target molecule;

(b) allowing substantially all of the target molecules in the first sample to bind with the aptamers;

(c) separating unbound aptamers from the first sample by contacting the sample of step (b) with immobilized ligands wherein the ligands bind to the unbound aptamers;

10 (d) recovering a second sample containing the aptamer bound to target molecules; and

(e) using a quantitative replicative procedure to determine a quantity related to the concentration of the aptamer in the second sample and therefore related to the

15 concentration of target molecules in the first sample.

2. A method according to claim 1, wherein the nucleic acid aptamer is selected from the group consisting of natural or synthetic single-stranded DNA, double-stranded DNA, single-stranded RNA, double-stranded RNA and chemical modifications thereof.

3. A method according to claim 1, wherein the target molecule is present in the sample at molar concentrations less than their dissociation constants with respect to the aptamers.

4. A method according to claim 1, wherein the target molecule is present in the sample at molar concentrations equal to or greater than their dissociation constants with respect to the aptamers.

5. A method according to claim 1, wherein the target molecule are low abundance molecules.

6. A method according to claim 1, where the target molecules include biological macromolecules.

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7. A method according to claim 6, wherein the biological macromolecules are selected from the group consisting of a protein, a lipid, a polysaccharide or combinations thereof.

10 8. A method according to claim 1, wherein the target molecules include small organic molecules.

9. A method according to claim 8, wherein the small organic molecules are selected from a group consisting of antibiotics, vitamins, steroids, and pesticides.

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10. A method according to claim 1, wherein the target molecules include inorganic molecules.

11. A method according to claim 10, wherein the inorganic molecules are metal.

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12. A method according to claim 11, wherein the metal is selected from a group consisting of metal ions, metal oxides, and metal complexes.

13. A method according to claim 1, wherein the first sample is obtained from an animal subject.

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14. A method according to claim 13, wherein the sample is selected from the group consisting of organ tissue, muscle tissue, bone tissue, connective tissue, fetal, placental, lymphatic tissue, vascular tissue, neuronal tissue.

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15. A method according to claim 1, wherein the sample is a biological fluid selected from blood, lymph, urine, sputum, joint including spinal fluid, and saliva.

16. A method according to claim 1, wherein the first sample is an environmental sample.

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17. A method according to claim 16, wherein the environmental sample is obtained from a plant, water, food beverages including milk, and industrial waste.

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18. A method according to claim 1, wherein the immobilized ligand is
10 immobilized on a support matrix selected from the group consisting of resins, beads, including magnetic beads, gels, cellulose and silica.

19. A method according to claim 1, wherein the immobilized ligand is immobilized on an affinity column.

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20. A method according to claim 1, wherein the quantitative replicative procedure is a quantitative polymerase chain reaction.

21. A method according to claim 1, wherein measuring the amount of aptamer
20 bound to the target molecule further includes denaturing the aptamer so as to separate the nucleic acid from the target molecules.

22. A method according to claim 21, wherein oligonucleotide primers are added to the sample after denaturing the aptamer from the target molecules.

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23. A method according to claim 22, wherein determining the concentration of aptamer includes determining a number of replicative cycles.

24. A method according to claim 6, wherein the target molecules are antibodies.

25. A method according to claim 24, wherein the target molecules include IgE.

26. A method according to claim 1, wherein the target molecules include a plurality of antibody molecules belonging to different subclasses characterized by a difference in the hypervariable region of the antibody.

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27. A method according to claim 1, wherein the target molecules are a subclass of an antibody having a characteristic hypervariable region.

28. A method according to any of claims 24-27, wherein the aptamer binds to a
10 constant region of the antibody and wherein the immobilized ligand is the constant region of the antibody for removing unbound aptamer from the sample.

29. A method according to claim 24, wherein the second sample contains antibody-bound aptamer, the second sample being divided into a plurality of aliquots, a first
15 aliquot of the second sample being assayed using a quantitative replicative technique to determine an amount of antibody in the first sample.

30. A method according to claim 29, further comprising:

- 20 (a) contacting a second aliquot of the second sample with an immobilized ligand for binding an antibody with a first hypervariable region; wherein the antibody with a first hypervariable region is one of the target molecules in the first sample;
- (b) recovering a third sample containing the aptamer bound to target molecules excluding the antibody with the first hypervariable region;
- 25 (c) assaying the aptamer concentration in the third sample using the quantitative replicative technique, so as to determine a difference in an amount of aptamer in the second sample and the third sample; and
- (d) obtaining a measure of an amount of the antibody with the first hypervariable region in the first sample from the difference.

31. A method according to claim 29, further comprising:

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- (a) contacting a plurality of aliquots of the second sample with an immobilized ligand wherein the ligand is immobilized by attachment to a substrate in a single chamber, or to multiple substrates wherein each substrate is contained in a separate chamber, each immobilized ligand having a specificity for an antibody with a different hypervariable site;
- (b) recovering a third sample containing the aptamer bound to target molecules excluding the antibody bound to immobilized ligand;
- (c) assaying the aptamer concentration in the third sample using the quantitative replicative technique, so as to determine a difference in an amount of aptamer in the second sample and the third sample; and
- 10 (d) obtaining a measure of the antibody with the hypervariable region in the first sample from the difference.

E 32. A method according to ^{any one of} claims 30 and 31, wherein the ligand is a specific antigen.

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33. A method according to claim 1, wherein the ligand is a reagent having the aptamer-binding characteristics of the target molecule.

^{any one of}
E 34. A method according to claim 30 and 31, wherein the antibody is IgE.

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35. A method for quantitatively evaluating *in vitro* an antibody response of a subject to an antigen, the method comprising:

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- (a) obtaining a sample from the subject and an aptamer for binding generally to all antibodies of a common class;
- (b) allowing substantially all of such antibodies in the sample to bind the aptamer in a common class mixture, so that the common class mixture includes antibody-aptamer complexes;

- (c) using antigen-antibody affinity binding to separate from an aliquot of the common class mixture those of the antibody-aptamer complexes having antibodies specific to the antigen, to produce (i) an antigen-specific antibody-aptamer complex and (ii) a difference mixture; and
- (d) using a quantitative replicative procedure to determine a quantity
- 5 related to the concentration of the antigen-specific antibody-aptamer complex relative to the concentration of the antibody-aptamer complexes in the common class mixture, and therefore related to the response of the subject to the antigen.

36. A method for quantitatively evaluating *in vitro* the response of a subject to a
- 10 plurality of different antigens, the method comprising:
- (a) obtaining a sample from the subject and adding an aptamer for binding generally to all antibodies of a common class in the tissue;
- (b) allowing substantially all of such antibodies in the sample to form a complex with the aptamer in a common class mixture, so that the
- 15 common class mixture includes antibody-aptamer complexes;
- (c) using antigen-antibody affinity binding to separate, from each of a plurality of aliquots of the common class mixture, those of the antibody-aptamer complexes having antibodies specific to a different one of the antigens, to produce with respect to each different antigen
- 20 (i) an antigen-specific antibody-aptamer complex and (ii) a difference mixture; and
- (d) using a quantitative replicative procedure to determine a quantity related to the concentration of each different antigen-specific antibody-aptamer complex relative to the concentration of the antibody-aptamer complexes in the common class mixture,
- 25 and therefore related to the response of the subject's tissue to each of the different antigens.

37. A method according to each of claims 35 and 36, wherein the sample is blood.

38. A method according to claim 35, wherein the antigen is an exogenous

allergen, and wherein the class of antibodies is IgE.

39. A method according to claim 36, wherein each of the antigens is a different exogenous allergen and the class of antibodies is IgE.

5 40. A method according to claim 35, wherein the antigen is associated with an infectious disease, and the class of antibodies is IgG.

10 41. A method according to claim 36, wherein each of the antigens is associated with an infectious disease, and the class of antibodies is IgG.

42. A method according to any of claims 35-41, wherein the quantitative replicative procedure utilizes a quantitative polymerase chain reaction procedure and includes determining the number of replicative cycles to produce a threshold indication.

15 43. A method according to claim 38, wherein the response of the subject is an allergic response to an allergen.

44. A kit for determining the allergic response of a subject to an allergen; comprising:

- 20 (a) an aptamer specific for a common class of IgE and a plurality of aptamers specific for individual allergens;
- (b) a plurality of reaction chambers for holding individual aliquots of a sample taken from the subject after binding the common class of antibody and removing unbound aptamer;
- 25 (c) an immobilized antibody for binding unbound aptamer of the common class prior to aliquoting the sample, and allergen for binding specific antibodies; and
- (d) means for quantitatively replicating the aptamers of the common class of IgE and for the aptamer-IgE complexes not bound to the allergen.

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